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ALPHA₁-ANTITRYPSIN DEFICIENCY

IN EARLY CHILDHOOD

Tomas Sveger

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α_1 -ANTITRYPSIN DEFICIENCY IN EARLY CHILDHOOD

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ALPHA₁-ANTITRYPSIN DEFICIENCY IN EARLY CHILDHOOD

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In collaboration with H. Ekelund.
- II α_1 -Antitrypsin deficiency, Pi genotype Z-, S- and M-.
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In collaboration with C.-B. Laurell and C.-G. Ljunggren.
- III Mass screening of newborn Swedish infants for α_1 -antitrypsin deficiency. *Am J Hum Gen*, 2:213-217, 1975.
In collaboration with C.-B. Laurell.
- IV Liver disease in α_1 -antitrypsin deficiency detected by screening 200,000 infants. *N Engl J Med*, in press 1976.
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INTRODUCTION

The aim of the present study

This paper concerns a prospective investigation of α_1 -antitrypsin (α_1 -AT) deficiency found on screening 200,000 infants. Special attention was given to the variation of protease inhibitors in newborn infants, to the screening procedure and to the prevalence of deficiency genes as well as to the occurrence of liver and lung disease during early life.

Serum protease inhibitors

The ability of human biologic fluids to inhibit proteolytic activity was recognized in 1897 by Camus and Gley, who noticed that serum had a considerable antitrypsin activity (1). Little progress was made until the last few decades, when the major trypsin inhibitor was localized to the electrophoretic α_1 -zone (2), and the inhibitors of proteolytic enzymes were isolated and characterized. The predominant protease inhibitors of human plasma are given in Table 1, and have recently been extensively reviewed (3).

Table 1. MAJOR PROTEASE INHIBITORS OF HUMAN PLASMA

Proteins	Mol.wt. daltons	Mean conc. g/l
α_1 -antitrypsin	55,000	1.35
α_1 -antichymotrypsin	70,000	0.5
Antithrombin III	65,000	0.3
α_2 -macroglobulin	725,000	2.0 ¹⁾
Inter- α -trypsin inhibitor	160,000	0.5 ¹⁾
C1 inhibitor	104,000	0.25
Antiplasmin	unknown	unknown

¹⁾ Levels significantly higher in children

α_1 -antitrypsin (α_1 -AT) is a glycoprotein with a molecular weight of 50-55,000 daltons and contains 12% carbohydrate (4). It is synthesized in the hepatocytes (5) and secreted into the plasma, where it constitutes a major protease inhibitor. Roughly 60% of the α_1 -AT occurs extravascularly. Many body fluids, including pulmonary secretions, tears and mother's milk, contain α_1 -AT (6). The half life of normal α_1 -AT is 5-6 days (7).

During inflammatory reactions, the level of α_1 -AT rises considerably (8). The synthesis of α_1 -AT is stimulated also by estrogens and can explain its elevation in women who are pregnant or using oral contraceptives (9). Several proteolytic enzymes, such as pancreatic trypsin and chymotrypsin (10), skin and synovial collagenases (11) and granulocytic proteases (12), are firmly bound by α_1 -AT which covers the active sites. The enzymes are accordingly inactivated in a 1:1 molar ratio (13). A transfer of the enzyme from the α_1 -AT complex to α_2 -macroglobulin and subsequent removal by the reticuloendothelial cells have been reported (12). The magnitude of the main function of α_1 -AT, the inhibition of proteases released from granulocytes, can be estimated from the fact that about 50 ml of granulocytes are produced daily, 5% of the dry weight contains elastase and collagenase (14). This corresponds to a release of about 1 g of proteases per day into the extravascular fluid. Unless sufficiently inhibited by α_1 -AT, these enzymes may digest elastin in the human pulmonary tissue.

α_2 -macroglobulin (α_2 -M) is a glycoprotein with a molecular weight of 725,000 daltons and contains 8% carbohydrate (15). Judging from animal experiments, about 20% is found extravascularly. The estimated half-life is 5-7 days (16).

The concentration of α_2 -M in early childhood is two-three times the normal adult value, to which it gradually falls during the first 25 years of life. In the nephrotic syndrome, α_2 -M is secondarily elevated. No deficiency has ever been revealed (3). Endopeptidases are irreversibly bound by α_2 -M. The complex formation results in such a conformational change that the molecule is recognized by the reticuloendothelial system and eliminated with-

in less than an hour. The binding of enzymes to α_2 -M does not affect the active site, and low molecular weight substrates can still be hydrolyzed. The basic function of α_2 -M probably is to inhibit endopeptidases in the extra- and intravascular compartments (17).

α_1 -antichymotrypsin (Achy) is a glycoprotein with a molecular weight of about 70,000 daltons and contains 27% carbohydrate (18).

The level of Achy increases within 8 hours of trauma (8). It is one of the acute phase proteins and responds rapidly to tissue injury, necrosis, and bacterial infections. Chymotrypsin was formerly thought to be bound to Achy, but its main inhibitor in vivo is α_1 -AT (19). In chronic bronchitis Achy is selectively concentrated in the bronchi and may be of importance to defend the mucus membranes of the bronchi against the proteolytic activity of the so-called cationic proteins released by granulocytes during phagocytosis (20, 21).

Inter- α -trypsin inhibitor (I- α -I) is a glycoprotein with a molecular weight of 160,000 daltons and contains about 8% carbohydrate (22).

Its concentration is increased during childhood (23). It is not known whether or in which way it varies in disease. Trypsin is bound and the acrosome of spermatozoa rapidly inhibited (24). The low molecular weight protease inhibitors of bronchial secretion are antigenically closely related to I- α -I, and it has been suggested that I- α -I might be a precursor of the secretory inhibitors (25).

Antithrombin III (AT III), with its molecular weight of 65,000, is the major inhibitor of thrombin (26). The inherited deficiency of AT III predisposes to recurrent thrombosis (27). The concentration of AT III is lower in women using oral contraceptives.

C1 inhibitor (C1 INH), a glycoprotein with a molecular weight of about 100,000 daltons, is an inhibitor of several specialized proteases such as C1 and kallikrein (28). Inherited deficiency of C1 INH is associated with hereditary angioneurotic edema (29). A functionally inactive C1 INH of normal quantity has been found in some patients with this disease (30).

Antiplasmin was recently found to be the major plasmin inhibitor (31). It is different from the other protease inhibitors and has so far not been further characterized.

The protease inhibitor (Pi) system

Starch gel electrophoresis at pH 4.9 (32) has revealed that α_1 -AT is genetically polymorphic. Antigen-antibody crossed electrophoresis has disclosed 2 major fractions and 6 minor ones. The electrophoretic mobility and serum concentration are controlled by a series of codominant alleles. The Pi (protease inhibitor) system contains more than 20 different alleles, which are designated by letters according to the electrophoretic mobility (33). The individual α_1 -AT alleles are called Pi^{M} , Pi^{Z} etc, and the resulting phenotypes MZ, MM etc. The Pi^{M} allele is the most common one and gives a normal amount of α_1 -AT in plasma. Of clinical interest are the variants that produce only an abnormally small amount. The Pi^{Z} allele produces an amount resulting in a serum concentration of approximately 15%, and the Pi^{S} allele of about 65% of the level produced by the normal Pi^{M} allele (34). A recently described rare allele, the Pi^- ("Pi null") allele, is seen in persons with no detectable α_1 -AT in serum (35). According to an international agreement (36) the phenotypes of presumably homozygous subjects (Pi MM, Pi ZZ etc) are designated Pi M, Pi Z etc, unless a family study has confirmed homozygosity and excluded the Pi^- allele.

The biochemical background of the α_1 -AT polymorphism is still obscure. Preliminary comparison of the sialic acid residues of M and those of Z protein has revealed that the latter contains one or two more residues less (37). It is known that glycoproteins with reduced sialic acid content are eliminated more rapidly from the circulation (38). Jeppsson has recently shown an amino acid substitution of glutamic acid by lysin in the Z protein (39). The molecular abnormality of the S protein has been identified and found to be a single change in the peptide map, a substitution of glutamic acid by

valine (40). Both substitutions are compatible with a point mutation in the genetic code that may explain the slow electrophoretic mobilities of the Z and S variants. The carbohydrates are attached to the same fragment but it is still uncertain how the amino acid substitution interferes with the completion of the carbohydrate chain (39).

Population genetics of α_1 -antitrypsin

The first α_1 -AT phenotype variants of clinical importance were detected by Eriksson and Laurell 1963 (41). Other phenotypes have been defined according to the mobility of the protein on acid starch-gel electrophoresis (32). Special attention has been given to the Pi phenotypes associated with α_1 -AT deficiency and correlated with lung emphysema and liver cirrhosis.

The most common gene in all population studies is Pi^M , with a frequency of about 0.9 or still higher (42). The reported frequencies of the deficiency alleles, Pi^Z and Pi^S , varie widely from country to country in Europe and reports from USA and Japan. The results of major population studies are given in Table 2. The Pi^Z allele is common in Sweden and Norway, less so in USA, still less so in France and has never been found in Japan. The frequency of the Pi^S allele is substantial in France, lower in USA, Norway and Sweden, and rare in Japan.

Table 2. THE Pi^Z AND Pi^S ALLELE FREQUENCIES IN DIFFERENT COUNTRIES

Country	Number of subjects	Pi^Z	Pi^S
Sweden (III)	200,000	0.026	unknown
Norway (43)	2,830	0.027	0.026
France (44)	1,281	0.006	0.08
USA (42)	2,287	0.013	0.034
Japan (45)	965	none	0.003

α_1 -antitrypsin deficiency and lung disease

About ten years ago Laurell and Eriksson noticed for the first time that the α_1 -globulin band was missing in the electrophoretic pattern of the serum from some patients with pulmonary emphysema (41). Some years later Eriksson published his classical monograph with descriptions of genetic, clinical and biochemical characteristics of α_1 -AT deficiency Pi ZZ and lung disease (46). The prevalence of α_1 -AT deficiency Pi Z among patients with emphysema varies depending upon selection criteria (47, 48, 49). As high a figure as 30% Pi Z individuals among younger patients with severe emphysema may be representative (48). Most of the patients in Eriksson's study were relatively young: The condition had been diagnosed below the age of 40 years in 60% and below 50 in 90% of the patients. Progressive shortness of breath with minimal cough or recurrent pulmonary infections were usually the first symptoms. In contrast with what is seen in ordinary emphysema, the main alterations develop in the basilar parts of the lung. Decreased perfusion in the dependent areas on lung scans appear early in the course of the lung disease. Pathologically most of the involved parts of the lung show panacinar emphysema (50).

Since 1965 more than 200 cases of chronic obstructive pulmonary disease (COPD) associated with α_1 -AT deficiency Pi ZZ have been described (47). But the risk of the α_1 -AT deficient individuals developing COPD during life cannot be predicted from these selected materials. Many of the published cases with an early onset COPD have heavy smoking histories that suggest smoking and air pollution as important additional factors. Kueppers and Black (47) reviewed non-smokers with α_1 -AT deficiency and traced 44 cases in the literature. The mean age at onset of clinical lung disease was 44 years, 10 years later than that of a large group Pi ZZ smokers. More than two thirds of the smokers had chronic bronchitis before 40 years of age compared with less than one tenth of the non-smokers. As the disease progresses, bronchitis and bronchiectasis may develop. The

rate of deterioration of lung function varies widely suggesting an influence of unknown factors. Patients may, however, live with their disease into the sixth and seventh decades and in non-smokers the course is often less malignant than hitherto assumed.

The association of other Pi variants with decreased α_1 -AT with COPD is less clear. The risk is probably much smaller for Pi SZ individuals (48, 51). A recent study of asymptomatic Pi Z and Pi SZ patients, however, (52), suggests that the risk of Pi SZ persons developing COPD may be substantial.

Most Pi ZZ persons show no pulmonary changes before puberty but some cases of COPD and α_1 -AT deficiency in children are on record (53, 54, 55). Some of these children had coexisting cystic fibrosis and asthma as the main causes of lung disease, and in one the onset of COPD had been preceded by measles pneumonia. According to Talamo (56) infants and children with idiopathic chronic pulmonary symptoms should be examined for α_1 -AT deficiency.

α_1 -antitrypsin deficiency and liver disease

Six years after the observation of the association between severe deficiency of α_1 -AT and lung disease, Sharp and coworkers (57) recognized the association between α_1 -AT deficiency and juvenile cirrhosis. The livers of the children with α_1 -AT and liver disease showed a wide variation of abnormalities ranging from mild peripheral fibrosis to severe aggressive hepatitis and cirrhosis. Biliary stasis is found especially in the initial cholestatic phase. Retrograde cholecystography often reveals bile duct hypoplasia of varying severity and a decrease in the luminal diameter of the intrahepatic bile ducts and sometimes also of the extrahepatic (23).

Specific abnormalities of the hepatocytes in Pi ZZ subjects, so called inclusion bodies, were noted in children (54) as well as in adults (58, 59). The material of the inclusion bodies was P.A.S.-positive, antigenically similar to α_1 -AT and on electronmicroscopy

found to be located in the rough endoplasmic reticulum (54). Both homozygotes and heterozygotes for the Pi^Z allele have more or less P.A.S.-positive material (59). Further analysis produced evidence that the inclusion bodies consisted of aggregated asialo antitrypsin (60). The difference was not only a complete lack of sialic acid, but also a relative deficiency of the other carbohydrate components in the liver compared with serum $\text{Pi ZZ } \alpha_1\text{-AT}$ (61). Our knowledge does permit the formulation of a hypothesis. The low level of $\alpha_1\text{-AT}$ with reduced half life may stimulate the synthesis of the abnormal Z protein. The synthesis proceeds with some difficulty from rough to smooth endoplasmic reticulum and part of the protein may accumulate after a few carbohydrates have been added. The part of the $\alpha_1\text{-AT}$ that accumulates never reaches the Golgi apparatus where sialyltransferase completes the final addition of sialic acid before secretion (62). The sialyltransferase activity was found to be normal in Pi ZZ children of this study, compared with Pi MM (63).

The initial clinical picture of neonatal hepatitis, found in about 10-20% of the Pi Z infants, may be followed by juvenile cirrhosis, which usually is a late complication (53, 54, 57, 64, 65, 66). A prospective study of the neonatal hepatitis syndrome in South-East England reported the frequency of neonatal hepatitis syndrome as 1/2000 while the combination of $\alpha_1\text{-AT}$ deficiency Pi Z and neonatal hepatitis was 1/14000 (67). The onset of the disease during childhood often starts during the first months of life. The typical symptoms are characterized by cholestatic jaundice, pale stools, hepatosplenomegaly and low weight gain. Bleeding problems may be prominent. The cholestasis usually disappears, but varying subclinical and biochemical abnormalities frequently persist. After a variable interval clinical signs of cirrhosis may appear, usually within 2-10 years (57). The natural history is, however, still fairly obscure, but the prognosis is not necessarily as bad as supposed (68, 69, 70). Almost all the children with $\alpha_1\text{-AT}$ deficiency and liver disease have been of phenotype Pi ZZ , but a few of the Pi SZ

phenotype have been described (71, 72). Of children with cirrhosis or chronic aggressive hepatitis and a history of neonatal hepatitis when first seen, about two thirds have α_1 -AT deficiency Pi ZZ (23). Some 10% of Pi Z individuals with no evidence of liver disease in early life develop cirrhosis later in life and the risk of hepatoma also seems to be increased (73).

PRESENT INVESTIGATION

Methods

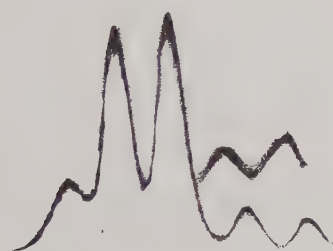
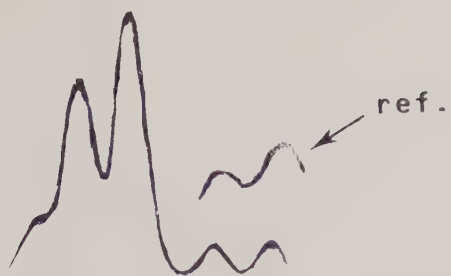
The concentrations of the protease inhibitors were determined in our study by the electroimmunoassay (74). α_1 -AT can be measured also by functional methods. The total trypsin and elastase inhibitory capacity give results in good accord with those obtained by immunochemical methods (75). So far no nonfunctional α_1 -AT has been found.

A standard pool of serum from 1,000 donors, mostly men, was regarded as 100%. This is roughly equivalent to 1.35 g α_1 -AT/l, 1.8 g α_2 -M/l and 0.5 g Achy/l.

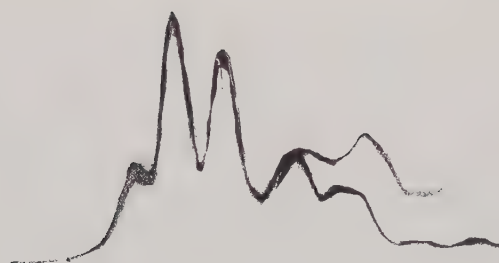
The initial Pi typing method was developed by Fagerhol and Laurell (32). The different techniques now available have recently been reviewed (76).

A less elaborate method was used at the laboratory, which is based on agarose gel electrophoresis (pH 5.15) followed by crossed immunoelectrophoresis (77). α_1 -AT is separated into five major bands and their mobilities are estimated relative to that of an internal reference, a carboamylated immunoglobulin L chain. The configuration and relative mobilities of the most recognized homozygous Pi phenotypes are illustrated in Fig. 1. The heterozygotes have a mixed pattern.

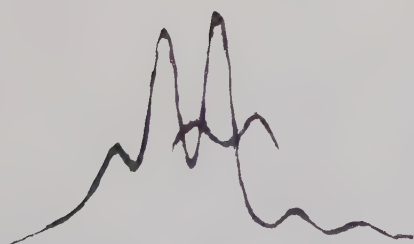
Pi MM



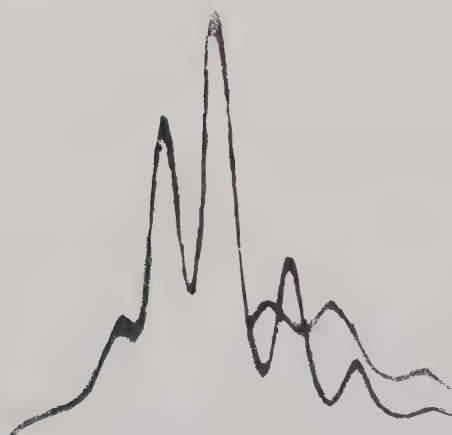
Pi SS



Pi MZ



Pi ZZ



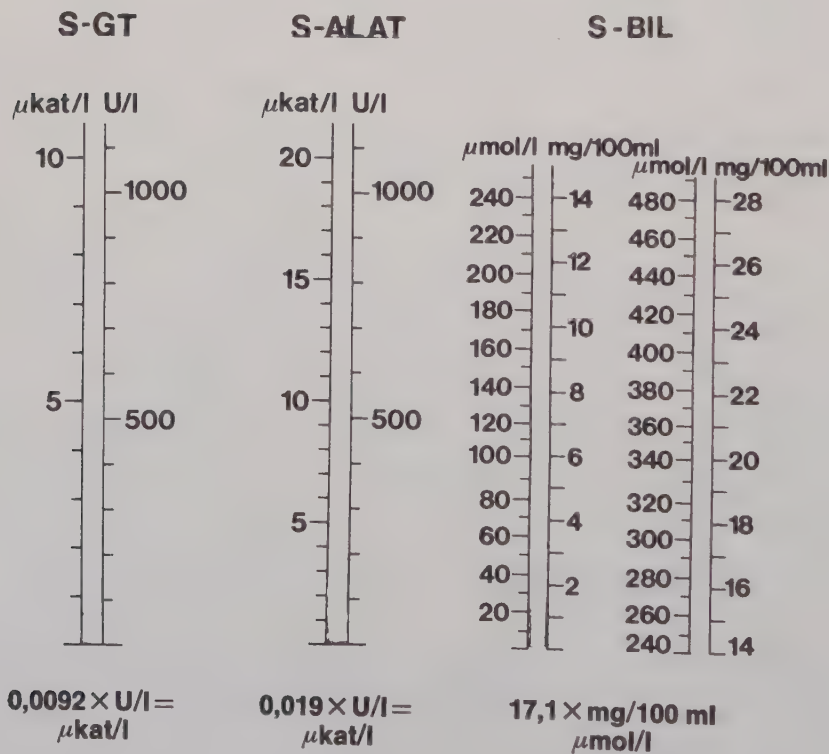
Pi SZ

Fig. 1. Typical patterns of the common Pi phenotypes.

Recently a new and quicker, though still accurate method for detecting the major Pi types has been developed and used by several investigators (78, 79, 80, 81). Electrofocusing in polyacrylamide gels with a pH range of 4 to 6, separates the different Pi bands in a single step. To overcome some of the technical problems special ampholytes have been introduced at our laboratory together with a thiol enrichment technique to make the interpretation more adequate (82). The electrofocusing technique has a far better resolution and is less time consuming than the others.

The α_1 -antitrypsin screening test has recently been described (83).

The concentration of α_1 -AT, Pi typing and glutamyl trans-peptidase (S-GT) level were determined at our laboratory and the determinations of bilirubin (S-Bil), alkaline phosphatase (S-ALP), alanine aminotransferase (S-ALAT, S-GPT) were made at different hospital laboratories with routine methods. The relation between I.U. and the SI system is given below for the liver tests.



Serologic examination for hepatitis B antigen and antibody, cytomegalovirus, toxoplasma, rubella, coxsackie and adenovirus were performed at the viral laboratories of the university clinics. Samples were obtained from the mother and on two occasions from the children when they were about 2 and 6 months old. Serum was examined for hepatitis B antigen by counterelectrophoresis or methods of approximately similar sensitivity (84).

Variation of protease inhibitors in fetuses and newborn infants (I)

The levels of α_1 -AT and α_2 -M during intrauterine life are well known (85). We found, like the previous workers, that the concentration of α_1 -AT in 14-24 week old fetuses was the same as in term infants. The α_2 -M level rises during intrauterine life from about 75% below the normal adult level during the 2nd trimester to 50% above at birth. The level of Achy, unknown before, was 2% of the adult normal in the fetuses. The synthesis of Achy is probably started and stimulated perinatally and the level increases rapidly during the first days to normal or above normal values.

The Evans group (86) found that the trypsin inhibitory capacity was low in infants with hyaline membrane disease. These original observations were confirmed by several workers and ourselves (Fig. 2). Achy was significantly lower in the infants with IRDS (idiopathic respiratory distress syndrome) when compared with the corresponding control preterm infants. The sequential analysis of α_1 -AT, α_2 -M and albumin indicated that after an initial decrease, the values rapidly rose towards normal levels. Achy showed no corresponding drop in concentrations and steadily increased from birth.

The hyaline membranes that develop within 6-8 hours of birth consist of cell detritus, red blood cells and plasma proteins including α_1 -AT (87). The tissue damage due to hypoxia and acidosis may cause an abnormal proteolytic activity (88) and consequently complex formation and elimination of α_1 -AT and α_2 -M. A generalized

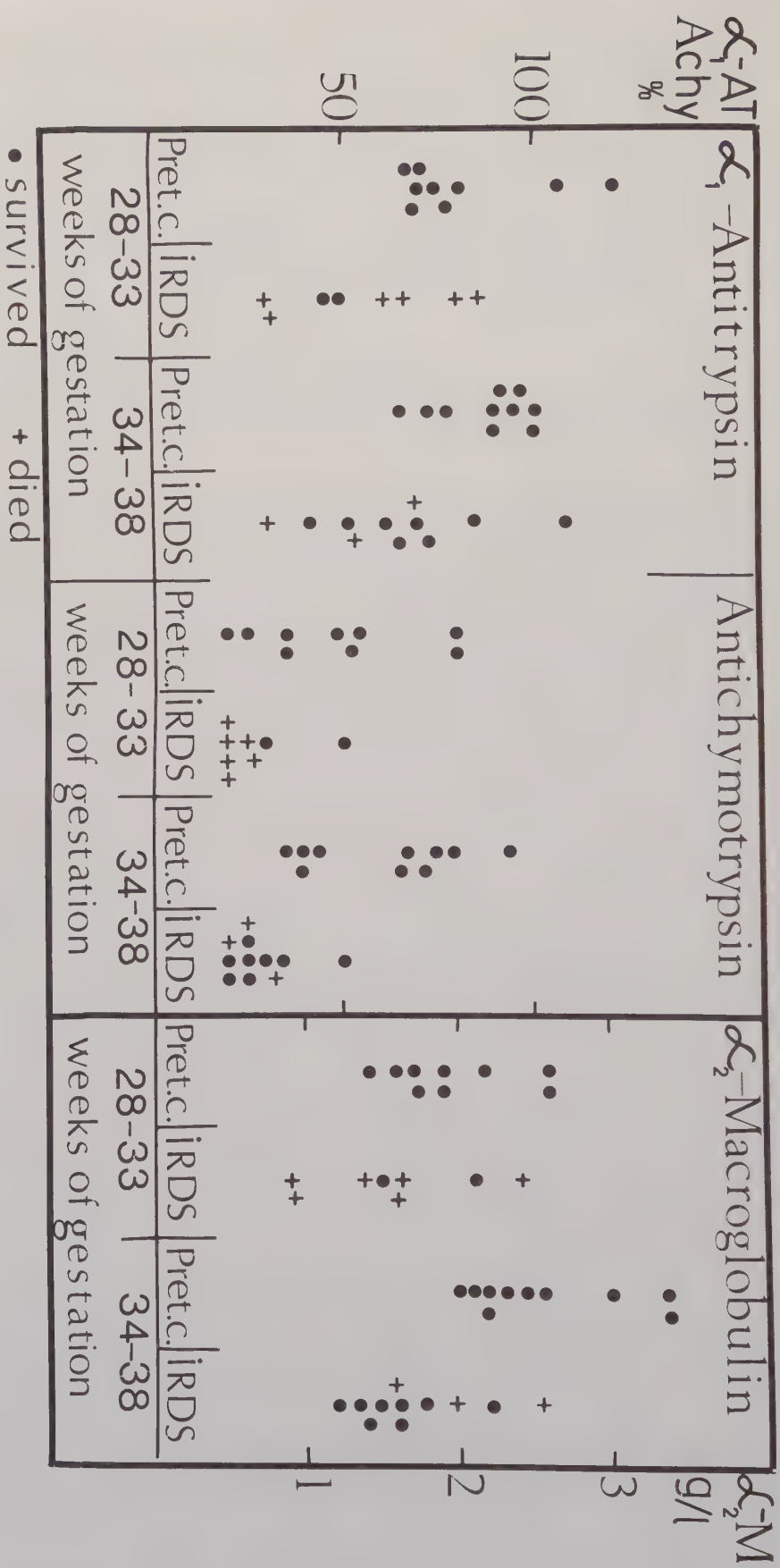


Fig. 2. The α_1 -antitrypsin, antichymotrypsin and α_2 -macroglobulin levels in IRDS compared with pre-term control infants of the same gestational age range. (100% α_1 -AT = 1.35 g/l and 100% Achy = 0.5 g/l).

immaturity of protein synthesis and hemodilution may also help to explain the low levels in babies with IRDS.

The prognostic importance of a low α_1 -AT value is uncertain because of the substantial overlapping of levels during the first hours after birth. Low Achy concentration perinatally seems to have as good a correlation with lung immaturity as α_1 -AT and α_2 -M. The extensive variation of the acute phase reactants in the IRDS infants during the first days of life make it unlikely that they could be useful as early reliable indicators of subclinical infections. The protease inhibitors studied were considerably increased in otherwise healthy newborns with premature rupture of the fetal membranes.

Evaluation of the α_1 -antitrypsin screening test (III)

The screening test for α_1 -AT deficiency developed by Laurell was used (83). A dried drop of blood on filter paper (sent to the PKU-laboratory for the Guthrie test) was eluted with saline. The eluate was used in a semiquantitative electroimmunoassay. The transferrin and α_1 -AT were of roughly the same concentration and solubility. The agarose gel contained antibodies to transferrin and α_1 -AT adjusted in such a way that the two peaks were equal in height. Since the precipitates differed in morphology, the two peaks were usually easily recognized. The transferrin level, which was used as internal standard, is more constant in newborn infants than in adults. The electroimmunoassay and measurement of the peaks are illustrated in Fig. 3.

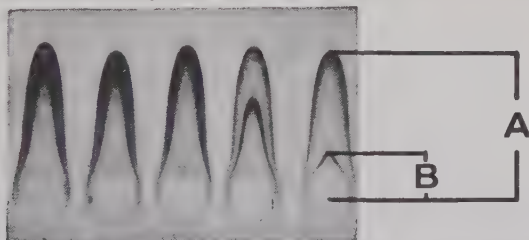


Fig. 3. Measurement of peaks in the electroimmunoassay. The heights of the transferrin (A) and α_1 -AT (B) peaks are measured from the center of the well. $B/A \times 100 = \% \alpha_1$ -AT of transferrin reference. Left to right: wells 1-3, normal; well 4, probably heterozygous; well 5, homozygous for α_1 -AT deficiency (III).

Infants with an α_1 -AT concentration of at most 30 % of the internal reference were preliminarily classified as Pi Z and those with a concentration between 31 and 40% as Pi SZ. Samples from all the infants with levels up to 40% of the reference were requested for Pi typing.

200,000 newborn infants were screened during a two year period. The Pi type and numbers of the infants with an α_1 -AT level of at most 30% and of 31% to 40% are given in Table 3. The mean concentrations of α_1 -AT at 2-4 months of age were $20 \pm 4\%$ for Pi Z infants and $40 \pm 5\%$ for Pi SZ. All Pi Z infants with a level below 2 SD were especially studied to exclude the Pi^- allele. Both parents were Pi typed in another 50 Pi Z families and only the mother in the majority of the remaining families. All the Pi Z infants whom screening had provisionally classified as Pi SZ had α_1 -AT in a concentration slightly above 30%. Six out of the 21 had clinically diagnosed liver disease that might have raised the α_1 -AT level, and/or decreased the concentration of transferrin. All Pi MZ and Pi M subjects who had less than 40% on the screen had levels within the normal ranges on later control. In 5 of these 12 subjects, the screen had been collected unusually late.

Table 3. THE PI TYPE OF THE INFANT WITH α_1 -ANTITRYPSIN LEVEL UP TO 40% OF THE REFERENCE, DETERMINED AFTER 200,000 NEWBORNS WERE SCREENED

Pi type	Level of α_1 -antitrypsin, % of reference		
	0 - 30%	31 - 40%	$\leq 40\%$
Pi Z	99	21	120
Pi SZ	3	45	48
Pi Z-	2	-	2
Pi S-	1	-	1
Pi MZ	1	6	7
Pi M	3	2	5
Not typed	11	8	19

The results reported after the first year (III) were confirmed after 200,000 infants had been screened. The screening procedure as well as the criteria used to select infants with homozygous α_1 -AT

deficiency are reliable. As a further control, serum from infants suffering from neonatal hepatitis syndrome were requested. None of those who had not been previously detected in the screening procedure had α_1 -AT deficiency Pi Z or SZ. Probably about two thirds of the total number of Pi SZ infants were excluded by the selected limit of 40% of the internal standard. This may be explained by factors that raise the Pi SZ α_1 -AT level like estrogen, intrauterine or neonatal infections, and premature rupture of the fetal membranes. The normal variation of the transferrin concentration in newborns may be further influenced by infections, prematurity and the age of the infant when the blood sample was collected (89).

Population genetics of α_1 -antitrypsin deficiency (II, III)

Our study was concerned mainly with the genes of major clinical importance. The Pi^Z allele frequency of 0.026 is almost identical with that of the one found among Swedish adults, 0.024(46). The Pi^S allele frequency in Sweden is not known and could not be calculated in our study. It is, however, probably close to that found in Norway, 0.026.

The Pi^- ("Pi null") gene was first found by Talamo et al. (35) in a young man with severe emphysema but with no clinical evidence of liver disease and no measurable α_1 -AT in the serum. The heterozygotes for Pi^- gene are indistinguishable from the homozygotes for the allele with expression in the Pi phenotype. The existence of the Pi^- gene, however, is suggested by the relatively low α_1 -AT level in individuals Pi M, S and Z. This is shown in the table and pedigree of a family with the Pi^- gene (Fig. 4, Table 4). We found 3 children, 2 Pi Z- and 1 Pi S-, who had an α_1 -AT level of about half that expected for the corresponding homozygous phenotype and in whom the existence of the Pi^- gene was proved by family study. Assuming that all Pi^- alleles in the children with Pi Z phenotype have been revealed the Pi^- allele frequency should be about 0.0002. The frequencies in Sweden of the

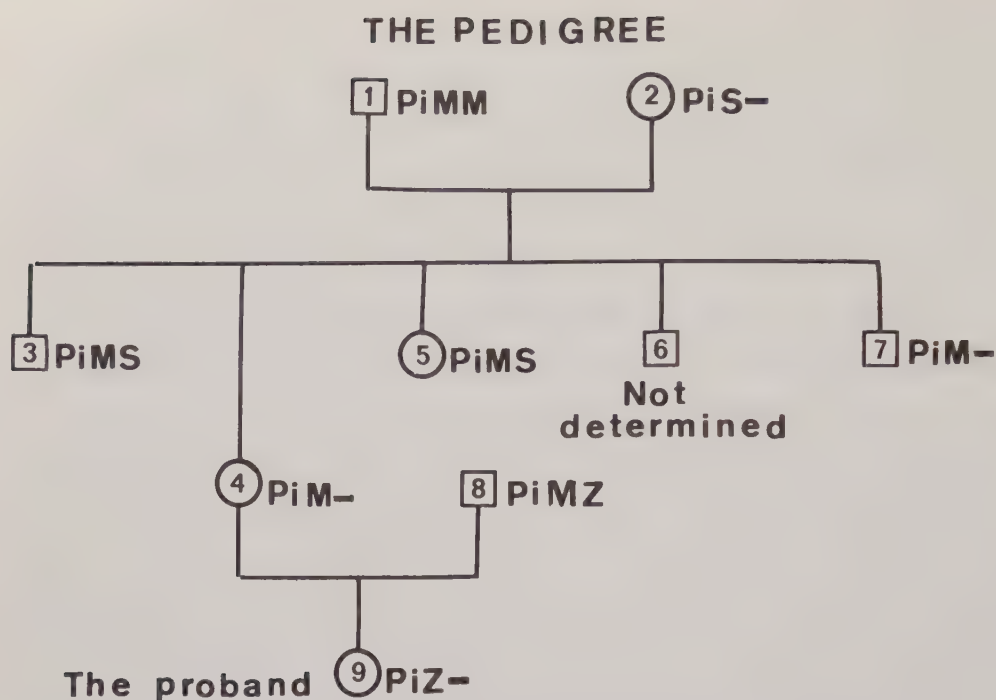


Fig. 4.

Table 4. SERUM α_1 -ANTITRYPSIN AND Pi TYPES IN THE PEDIGREE FAMILY

Pedigree number	Sex	Age	Serum α_1 -antitrypsin %	Pi type
1	M	64	115	MM
2	F	56	37	S-
3	M	30	90	MS
4	F	28	50	M-
5	F	27	90	MS
6	M	23	100	unknown
7	M	17	65	M-
8	M	28	60	MZ
9	F	0.25	11	Z-
		0.5	10	

Table 5. THE APPROXIMATE FREQUENCIES OF THE Pi PHENOTYPES THAT CAUSE α_1 -ANTITRYPSIN DEFICIENCY IN SWEDEN AND ESTIMATED CONCENTRATION OF α_1 -ANTITRYPSIN

Pi type	Frequency	Approximate level of α_1 -antitrypsin %
Pi - -	1/25 000 000	0
Pi Z -	1/100 000	10
Pi ZZ	1/1500	20
Pi S- ¹⁾	1/100 000	30
Pi SZ ¹⁾	1/750	40

¹⁾ The Norwegian PiS frequency is used

The high Pi^Z gene frequency in Scandinavia suggests that the mutation initially occurred in this part of the world. The fact that this gene is so often associated with severe disease has given rise to considerable speculation. The levels of several protease inhibitors, including α_1 -AT, in human cervical mucus are low. They fall to extremely low values at midcycle, i.e. at the time of ovulation and possible fertilization (90). Acrosin, the proteolytic enzyme of the acrosome (91), is essential for the penetration of the zona pellucida of the ovum and facilitates the penetration of the sperm through the cervical mucus. α_1 -AT inhibits acrosin. Theoretically, alleles associated with low levels of α_1 -AT may facilitate fertilization and the perpetuation of the Pi alleles that give reduced levels of α_1 -AT.

α_1 -antitrypsin deficiency and liver disease in children 0-3 years of age (IV)

Our investigation is the first prospective study of an unselected population of Pi ZZ and Pi SZ children. Almost all Swedish newborn infants born within a two year period were screened for α_1 -AT deficiency. The 200,000 infants screened, those found to have less than 40% as much α_1 -AT as a normal reference, were requested for Pi typing. A standardized report including information about any lung and liver disease of the parents, siblings and other close relatives was obtained from the pediatricians, as well as details about the pregnancy, delivery and perinatal period. The α_1 -AT deficient children were re-examined at about 1/4, 1/2, 1 and 2 years of age. The Pi type and sex of the children followed up are given in Table 6. The clinical results for the 0-1/2 year old children will soon be published (IV). The children were divided into different groups according to the clinical symptoms and laboratory results. The characteristics of the groups are summarized in Table 7.

Table 6. INFANTS WITH α_1 -ANTITRYPSIN DEFICIENCY: NUMBER, Pi TYPE, AND SEX

Total number screened	200,000		
Number Pi Z	120	(671, 0 49)	
"- Pi SZ	48	(0 25, 0 23)	
"- Pi Z-	2	(0 2)	
"- Pi S-	1	(0 1)	
Number not Pi typed	19	(0 9, 0 10)	

Table 7. DIAGNOSTIC CRITERIA OF THE CLINICAL GROUPS

Group	Diagnostic criteria
I A	Cholestatic jaundice, pale stools, hepatosplenomegaly, failure to gain weight at < 4 months of age.
I B	Clinically asymptomatic, conjugated S-Bil > 2.0 mg/100 ml at 2-3 months of age.
II	One or more of the symptoms: hepatomegaly, splenomegaly, late umbilical bleeding, failure to gain weight, history of unverified prolonged jaundice.
III	Clinically asymptomatic. Children with increase of S-GPT, S-GT and S-Bil $\leq$ 2.0 mg/100 ml are included.
IV	Not Pi typed. Screen level $\leq$ 40% of the reference level.

Liver tests were analyzed in 22 healthy children to determine the normal range of variation for children. On the basis of the results the following upper normal limits for children 1/4-2 years of age were chosen:

Total S-Bil	1.0	mg/100 ml	(17 μ mol/l)
S-ALP	1000	U/l	(16.7 μ katal/l)
S-GPT	50	U/l	(1.0 μ katal/l) and
S-GT	130	U/l	(1.2 μ katal/l)

Table 8. THE PERINATAL HISTORY OF THE INFANTS WITH α_1 -ANTITRYPSIN DEFICIENCY

	GROUPS				
	I A	I B	II	III Pi Z	III Pi SZ
Number of boys	6	4	5	56	25
	} 9	} 5	} 8	} 98	} 48
Number of girls	3	1	3	42	23
Heredity for liver disease ¹⁾	3	0	0	1	0
Complications of gestation ²⁾	1	1	3	7	5
Complications of delivery	0	0	0	4	1
Preterm	0	0	0	8	3
Small for gestational age (SGA)	4	3	3	17	2
Asphyxia ⁴⁾	0	0	0	4	1
Hyperbilirubinemia ⁵⁾	2	0	2	4	4
Symptomatic hypoglycemia	1	0	1	1	0
Septicemia	1	0	0	1	0

¹⁾ Neonatal hepatitis syndrome, hepatocarcinoma or cryptogenic cirrhosis.

²⁾ Toxicosis, benign cholestatic jaundice of pregnancy, bleeding.

³⁾ Abnormal position, bleeding, umbilical cord prolapse.

⁴⁾ Intra- extrauterine asphyxia. Apgar score > 8 p. at 5 minutes.

⁵⁾ Total bilirubin > 15 mg/100 ml the 1st week.

Table 9. CLINICAL HISTORY, EXAMINATION AND LIVER TESTS OF THE INFANTS IN GROUP I AND II

	GROUP I A	GROUP I B	GROUP II
Total number	9	5	8
Age of onset	4	8	8
median(range)weeks	(1 - 6)	(4 - 12)	(4 - 24)
Hepatomegaly	5	0	2
Splenomegaly	4	0	2
Failure to gain weight	9	0	2
Maximal recorded levels of:			
S-Bil median (range)	8.5	3.2	0.6
mg/100 ml	(4.7 - 12.0)	(2.0 - 4.2)	(0.2 - 1.5)
S-GPT median (range)	50	50	55
U/l	(15 - 100)	(24 - 90)	(44 - 265)
S-GT median (range)	640	717	251
U/l	(258 - 2850)	(216 - 1252)	(194 - 1225)

The following clinical and biochemical results were obtained during the first three years of life:

GROUP I A consisted of infants with prolonged obstructive jaundice. These patients would have been recognized without α_1 -AT screening. The case histories of the 9 children are presented below and the perinatal histories and clinical and laboratory results summarized in Tables 8 and 9. All had α_1 -AT deficiency Pi Z.

Case 1. E.K., a girl, was the 2nd child of healthy Pi MZ parents. Also her brother, Pi ZZ, had suffered from prolonged jaundice, not further studied, and when last seen at 3 1/2 was apparently healthy. The mother had bacteriuria in the 5th month of gestation. Delivery was normal and the girl weighed 2,900 g. In the 5th day total S-Bil reached a maximum of 18 mg/100 ml. At the age 3 weeks, she had increasing jaundice, passed pale stools and did not gain weight. Conjugated S-Bil 12 mg/100 ml, S-GT 764 U/l and S-GOT 123 U/l. A blood culture was positive and indicated coli septicemia, which was successfully treated with ampicillin. After about 3 weeks S-Bil gradually fell and at 2 months S-Bil was 1.7 mg/100 ml, S-ALP and S-GPT normal, and weight gain adequate. Viral serology was negative. Controls at 1/4, 1/2, and 1 year of age were clinically normal with a liver and spleen size normal. S-Bil was 0.5 mg/100 ml, S-ALP normal, S-GPT 49 U/l at 1 year, and S-GPT 285 U/l at 1 1/2 years and 31 U/l, 2 months later. At the last check-up, 2 years, she was apparently healthy, S-Bil 0.7 mg/100 ml, S-ALP normal and S-GPT 67 U/l.

Case 2. G.J., a boy, was the 2nd child of healthy Pi MZ parents. His sister was Pi MM and healthy. Pregnancy and delivery were uncomplicated; b.w. 2,240 g in the 38th week of gestation. On the 3rd day S-Bil reached a maximum of 12.2 mg/100 ml. He was admitted at 2 months of age because of jaundice, hepatosplenomegaly and poor weight gain. Conjugated S-Bil 5.8 mg/100 ml, S-GT 258 U/l and S-GPT within normal limits. The boy gradually improved and at 4 months he was clinically healthy with normal S-Bil, S-ALP and S-GPT, but the hepatosplenomegaly persisted. Viral serology was negative. Control examination at 1/2, 1 and 2 years of age revealed nothing remarkable except hepatomegaly. At 3 years of age he had suffered from persisting cough for 1 year and the liver was enlarged and palpable, 6 cm below the costal arch. Clinical signs of liver cirrhosis had appeared with spiders and palmar erythema. S-Bil, S-GPT and prothrombin time were normal.

Case 3. N.J., a girl, was the 3rd child of a healthy Pi MZ father and a Pi MZ mother with asthma and eczema. A two year older brother, Pi ZZ, had prolonged obstructive jaundice and he is now 3 years old and apparently healthy. The sister, Pi ZZ, is now 9 years old and she has always felt well. The b.w. of N.J. was 2,360 g and gestation and delivery had been normal. On the 6th day total S-Bil was 9.0 mg/100 ml and unconjugated. At 6 weeks she was admitted with jaundice and poor weight gain. Conjugated S-Bil 47 mg/100 ml but S-ALP and S-GPT normal. After 3 weeks she improved and at 3 months she was apparently healthy.

Viral serology was negative. Further check-ups at 1/2, 1 and 2 years revealed nothing remarkable.

Case 4. D.O., a boy, was the 2nd child of healthy Pi MZ and Pi SZ parents. A sibling, not Pi typed was healthy, as was a Pi ZZ cousin, included in the screening procedure. D.O. was born in the 38th gestational week, b.w. 3,110 g after a normal gestation and induced delivery because of low estrogen levels indicating placental insufficiency. On the 5th day S-Bil was 15.1 mg/100 ml but fell to 13.1 mg/100 ml by the 10th day. A short period with clay-coloured stools between 1 and 2 weeks of age was noticed. At 6 weeks he was admitted because of jaundice and poor weight gain. Conjugated S-Bil 5.8 mg/100 ml, S-GT 640 U/l and S-GPT 34 U/l. Bacteriuria was suspected and the boy was treated with ampicillin. Blood culture and viral serology were negative. The jaundice gradually disappeared, S-Bil at 2 months 3.6 mg/100 ml, and at 3 months normal. Check-ups at 1/2, 1 and 1 1/2 years have shown slightly increased S-GPT, but otherwise nothing remarkable.

Case 5. F.C., a boy, was the 1st child of healthy Pi MZ parents. His mother had had pre-eclampsia, the delivery was normal in the 40th gestational week and b.w. 2,500 g. Five hours after birth he had a short attack of cyanosis and when 1 day old he had convulsions due to hypoglycemia that relapsed for three days despite glucose and cortisone treatment. Blood and urine cultures were negative. When about 1 week he had jaundice, hepatosplenomegaly and mild edema. Conjugated S-Bil 5.0 mg/100 ml but S-GPT and S-ALP were normal. Bacteriuria was suspected in another culture and ampicillin was given. During the 2nd week S-Bil increased and S-ALP and S-GPT became slightly abnormal. At 3 months of age the boy had improved and S-Bil was 1.2 mg/100 ml and S-ALP and S-GPT normal. A systolic murmur heard at about 2 months was due to an aneurysm of the Botalli duct and coarctation of the aorta. The heart malformations were successfully corrected at about age 3 months. At the last check-up when the boy was one year old, he had slight right hemiparalysis, and S-ALP and S-GPT were increased.

Case 6. F.J., a boy, was the 2nd child of healthy Pi MZ parents. A sibling was healthy but not Pi typed. An uncle had died from an unspecified cirrhosis and another uncle, had had emphysema in young adulthood. The gestation and delivery were normal and b.w 3,970 g. At 12 days of age the child was admitted to hospital because of jaundice, vomiting and decrease in weight. Total S-Bil 8.0 mg/100 ml, about half conjugated, and S-ALP and S-GPT normal. Conjugated S-Bil reached a maximum (7.0 mg/100 ml) at 3 weeks. During the next few months, jaundice disappeared and the boy improved clinically. Check-ups at 4, 5 and 8 months of age revealed evidence of upper respiratory tract infection. At 1 and 1 1/2 years, he was apparently healthy, and both S-ALP and S-GPT were within normal ranges.

Case 7. J.R., a boy, was the 1st child of healthy parents, mother Pi MZ. The gestation and delivery were normal and b.w. 3,450 g. On the 4th day S-Bil 8.7 mg/100 ml. At 1 month, the boy was admitted because of increasing jaundice, no weight gain and clay-coloured stools. Conjugated S-Bil 9.6 mg/100 ml. S-GT 445 U/l and S-GPT 75 U/l. After 3 weeks the boy improved and at 2 1/2 months he was apparently healthy,

but conjugated S-Bil 2.4 mg/100 ml. Viral serology was negative. Check-ups at 1/4, 1/2 and 1 1/2 years of age have shown a persistent rise of S-ALP and S-GPT, but clinically the boy is healthy.

Case 8. N.T., a boy, was the 3rd child of healthy Pi MZ parents. 2 siblings, Pi MM and Pi MZ, were healthy. The gestation and delivery were normal, b.w. 3,480 g. At 5 weeks, he had not gained in weight since birth, was jaundiced and had clay-coloured stools. Conjugated S-Bil 9.0 mg/100 ml, S-GT 2,850 U/l and S-GPT 100 U/l. During the next few weeks the liver and spleen increased considerably. At age 2 1/2 months S-Bil 3.0 mg/100 ml and S-ALP and S-GPT slightly increased. Viral serology was negative. At 1 and 1 1/2 years, the boy was healthy with normal-sized liver and spleen but the S-GPT was slightly abnormal.

Case 9. L.M., a girl, had healthy parents and a brother, not Pi typed. The gestation and delivery were normal and b.w. 2,700 g. At 1 month she was hospitalized because of jaundice, suspected of malnutrition and hepatosplenomegaly. Conjugated S-Bil 5.3 mg/100 ml, and S-GPT 100 U/l. The jaundice subsided and at 3 months she still had hepatosplenomegaly and S-GPT 145 U/l, but S-Bil, S-ALP and the clinical findings were otherwise normal. At 1 year she had improved further but the liver and spleen were still slightly enlarged.

GROUP I B The 5 Pi Z infants of this group had biochemical signs of cholestasis, which would probably not have been found unless specifically examined for the condition. Their case histories are given below and the perinatal and clinical histories and laboratory results are summarized in Tables 8 and 9.

Case 10. A.J., a boy was the first child of healthy Pi MZ parents, no prenatal problems, b.w. 2,900 g. At 2 months of age, he was healthy, but S-Bil was 3.2 mg/100 ml and S-GT 698 U/l. At 3 months S-Bil was normal and S-GPT 90 U/l. Recurrent medial otitis was the main clinical problem during the first 2 1/2 years. At 2 years, however, liver tests during bronchitis showed S-GPT 694 U/l, which was 42 U/l one month later. The liver tests were normal at the 2 1/2 year check-up.

Case 11. S.A., a girl, was the first child of healthy parents not Pi typed. She was born in the 39th gestational week, b.w. 2,200 g, after an uncomplicated pregnancy. At 3 months of age, she was healthy, but S-Bil was 2.6 mg/100 ml, S-GT 735 U/l and S-GPT 50 U/l. At 1/2 years S-ALP was abnormal. Subsequent check-ups at 1, 1 1/2 and 2 years of life revealed nothing remarkable.

Case 12. S.A., a boy, was the 4th child of healthy parents. Neither the parents nor the healthy siblings have been Pi typed. B.w. was normal. He was slightly jaundiced at the 3 month check-up and S-Bil was 3.4 mg/100 ml, and S-GPT 84 U/l. The following controls at 1/3, 1, 1/2 and 2 years revealed nothing remarkable except an elevated S-GPT, 123 U/l, at 1 1/2 years.

Case 13. T.J., a boy, was the second child of a healthy father, and a mother who had chronic nephritis. The sibling was healthy. None of them have been Pi typed. During the gestation the mother developed toxicosis. The delivery was normal and b.w. 2,670 g. At 3 months of age the boy was healthy, but S-Bil 2.0 mg/100 ml, S-GT 1,252 U/l and S-GPT 35 U/l. Viral serology was negative. At 1 1/2 years the boy was healthy, but the liver felt hard and the spleen enlarged. S-Bil 1.1 mg/100 ml, S-ALP increased and S-GPT 513 U/l. At about 2 years he had a persistent cough, splenomegaly and the development of cirrhosis was suspected.

Case 14. K.M., a boy, was the second child of healthy Pi MZ parents. A sister, not Pi typed, has eczema. B.w. 2,840 g. At age 6 weeks, he had jaundice, S-Bil 4.2 mg/100 ml and S-GPT 24 U/l, but S-Bil, S-ALP and S-GPT were normal at the check-up 3 weeks later, while S-GT was 216 U/l. The clinical check-ups at 1/2 and 1 year revealed nothing remarkable, but S-GPT 141 U/l and 193 U/l respectively.

GROUP II Liver disease without jaundice was suspected clinically in 8 children, all Pi Z. The perinatal and clinical histories and the results of the liver tests are given in Tables 8 and 9, and the case histories are presented below.

Case 15. J.G., a boy, was the 1st child of healthy parents, mother Pi MZ. Delivery was induced due to the long gestation, 44 weeks, and b.w. 2,870 g. On the second day he was shaky and hypoglycemic. At 2 months the liver was hard, S-Bil 1.5 mg/100 ml, S-ALP increased, S-GT 194 U/l, and S-GPT 53 U/l. At 5 months he was clinically normal, S-Bil and S-ALP normal and S-GPT 80 U/l. At the 1 1/2 year check-up the liver was hard again, S-Bil and S-ALP normal, but S-GPT 130 U/l. At 2 years the boy was apparently healthy and S-GPT 60 U/l.

Case 16. F-M.A., a girl, was the second child of healthy Pi MZ parents. Benign cholestatic jaundice complicated the pregnancy. B.w. 2,860 g. At 1 month she was hospitalized because of assumed pyloric stenosis with vomiting and aspiration. X-ray of the gastrointestinal tract, 'metabolic screen' and lumbar puncture revealed nothing abnormal. At 2 months she was clinically healthy, S-Bil 1.2 mg/100 ml, S-GT 1225 U/l and S-GPT 66 U/l. At 1 year she was asymptomatic, but S-GPT 156 U/l and at 2 1/2 years apparently healthy with normal S-Bil and S-ALP and S-GPT 76 U/l.

Case 17. H.C., a girl, was the first child of a healthy Pi MZ mother and Pi SZ father with recurrent bronchitis. A brother, Pi SZ, was healthy. Benign cholestatic jaundice complicated the pregnancy. B.w. 3,700 g. She vomited fairly often during the first month and weight gain was poor. At 3 months she had splenomegaly and S-GT was 200 U/l. Viral serology was negative. At 6 months of age she was healthy, S-Bil and S-ALP normal and S-GPT 50 U/l. Neither clinical nor laboratory examinations showed any abnormalities at 1 and 2 years.

Case 18. L.M., a boy, was the first child of a healthy Pi SZ mother and a Pi MZ father who had recurrent bronchitis. B.w. 3,440 g. He was healthy at 2 months but had hepatosplenomegaly with S-GT 590 U/l. At 6 months the liver was still slightly enlarged, conjugated S-Bil 1.2 mg/100 ml, S-ALP and S-GPT normal. At 1 year he was healthy and S-GPT 69 U/l and at 2 years both the findings at clinical and laboratory examinations were normal.

Case 19. N.S., a girl, was the third child of healthy Pi MZ parents. Her siblings were also Pi MZ and well. B.w. 3,560 g. Exchange transfusion due to incompatibility of the ABO system and hyperbilirubinemia was done twice. The maximal S-Bil recorded was 16.7 mg/100 ml, which decreased to 5.2 mg/100 ml the second week. At 2 months transfusion was repeated because of anemia, S-Bil was 2 mg/100 ml, S-ALP increased, S-GT 260 U/l and S-GPT 148-265 U/l. At 8 months she had a urinary tract infection with considerable albuminuria. At 1 and 2 years she was apparently healthy.

Case 20. S.C., a boy, was the second child of a healthy Pi MZ mother and Pi MZ father with psoriasis. The brother, Pi MM, was well. Benign cholestatic jaundice complicated the pregnancy. B.w. 2,320 g in the 38th week. At 2 months of age he was healthy, S-Bil 1.3 mg/100 ml, S-GT 228 U/l and S-GPT 55 U/l. At 9 months the liver was enlarged, S-Bil and S-ALP normal, S-GPT 137-105 U/l. He was quite healthy at the 1 and 3 year check-ups, but S-GPT was 143 U/l at 1 year.

Case 21. T.R., a boy, whose mother, Pi MZ, had recurrent urticaria. Two brothers, not Pi typed, had eczema and asthma. B.w. 3,500 g. He had S-Bil 16.8 mg/100 ml on the third day and was clinically jaundiced about 1 1/2 months. At the 2 month check-up, conjugated S-Bil 1.5 mg/100 ml, S-ALP increased, S-GT 251 U/l and S-GPT 44 U/l. Viral serology was negative. At 1/4, 1/2 and 1 year he was apparently healthy. S-GPT was 74 U/l at 1/2 year.

Case 22. N.M., a boy, was the third child of a healthy Pi MZ mother and a Pi MZ father with recurrent bronchitis. The siblings with unknown Pi type were healthy. B.w. 4,010 g. At 1 month he was hospitalized because of bleeding from the umbilicus and had a severely prolonged prothrombin time. At about 1/2 year S-Bil and S-ALP were normal and S-GPT 106 U/l. He was apparently healthy at the 1/2 and 1 year check-ups.

The children in GROUP III had no clinical symptoms of liver disease. The results of the laboratory analyses at 1/4, 1/2 and 1 year are given in Fig. 5. The following Pi Z and SZ cases with other abnormalities not reported before were found.

Case 23. H.P., a Pi ZZ girl, was the 2nd child of a Pi MZ mother with asthma and Pi MZ father with eczema. A Pi ZZ brother was healthy. The 2 month check-up revealed nothing remarkable. She later developed exanthema and asthma after eating cow's milk formula, S-GPT was 100 U/l. Cow's milk was replaced by a soybean formula. At 7 months she still had recurrent asthma and eczema, and S-GPT was 123 U/l. Two months later she had a severe anaphylactic shock with brain damage and a high reagin titer was found not only

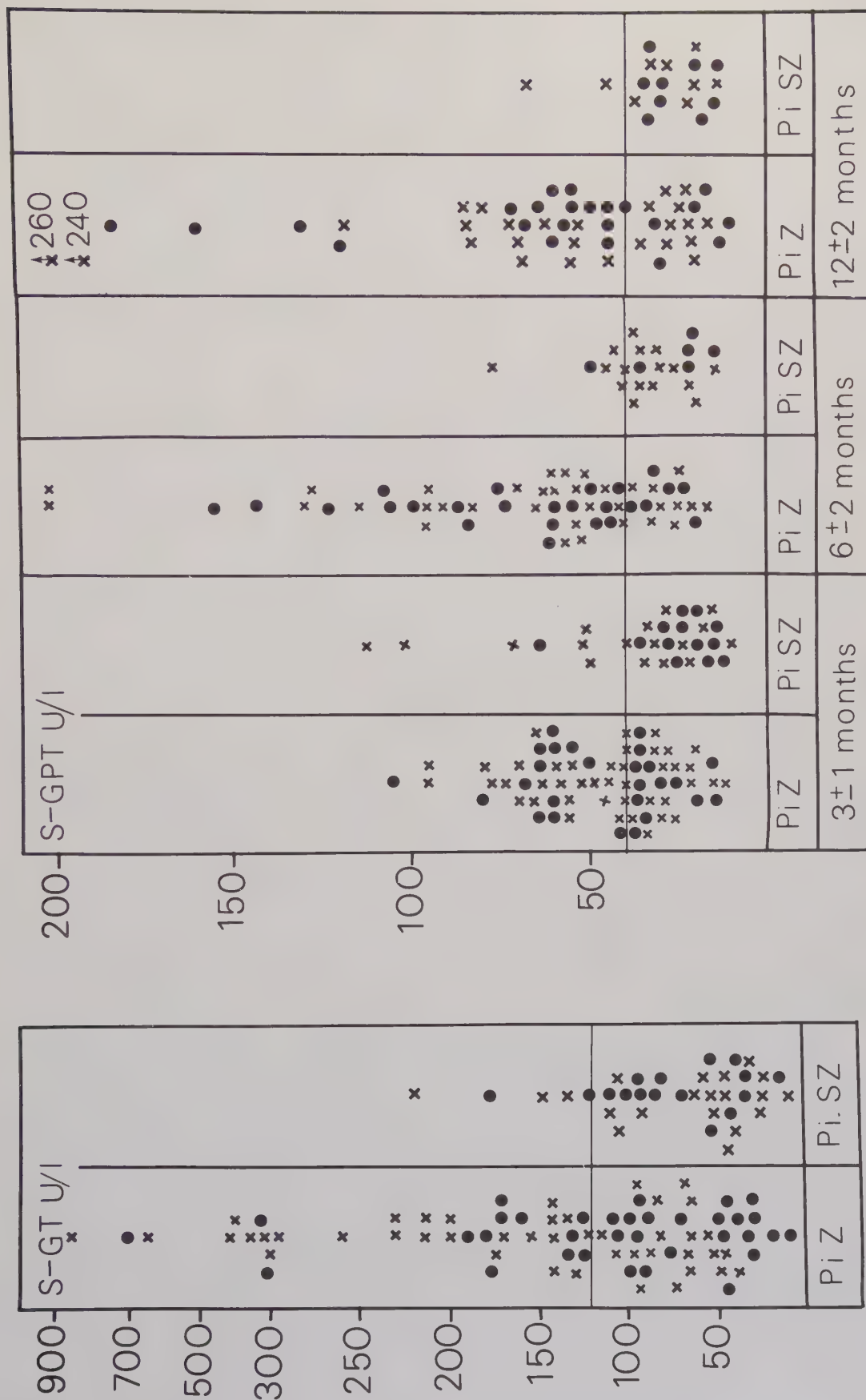


Fig. 5. Group III. The distribution of S-GT and S-GT at 1/4, 1/2, 1 and 1/4 years respectively. The line denotes the approximate upper normal limit. (● Girls, x Boys)

for cow's milk, but also for soybeen proteins.

Case 24. K.F., a Pi Z girl, was quite healthy until 2 1/2 years of age, when she got morbilli. One week after the first symptoms had started, the fever increased again and she had diarrhoea and vomiting. Septicemia was suspected after another 3 days and antibiotics were given. 3 days later peritonitis was suspected and laparotomy revealed a perforative appendicitis. One week later she was re-laparotomized because of assumed abscess formation but the only abnormality found was enlargement of the liver, almost white with fat. During the initial stage of acute morbilli S-Bil was 1.5 mg/100 ml and S-GPT 100 U/l. 2 months later she was healthy again, S-Bil 0.3 mg/100 ml, S-ALP normal and S-GPT 50 U/l. 3 months after the acute episode the liver was of normal size, S-Bil and S-ALP normal and S-GPT 81 U/l.

Case 25. L.N., a Pi ZZ boy, was born in the 31st gestational week, b.w. 1,080 g. The pregnancy was complicated by severe toxico-sis. He had transient asphyxia and hypothermia. On the third day he developed septicemia which was successfully treated. Despite the severely complicated pre- and perinatal period, he showed no signs of liver disease and check-ups including liver tests at 1/4, 1/2 and 1 year of age revealed nothing remarkable.

Case 26. N.S., a Pi ZZ girl, with healthy Pi MZ parents, was only 43 cm long at birth, b.w. 3,350 g. She had congenital chondrodystrophy.

Case 27. B.G., a Pi SZ girl, with healthy parents and sibling, was born after a pregnancy complicated by toxicosis. B.w. 3,440 g. At 2 years of age she had monoarthritis of the knee joint with re-current hydrops during the last 9 months.

Case 28. S.G., a Pi SZ boy, was the 1st child of healthy parents. B.w. 3,810 g. Appeared normal until 3 days after the 3 month check-up, when the child was found dead in his bed. At autopsy, no abnormalities were found and the diagnosis was "sudden unexpected infant death".

Case 29. B.M., a Pi SZ boy, has a severe malformation of the right hand with a short middle hand and no fingers.

The children not Pi typed, GROUP IV, all but 2 have so far been apparently healthy at the ordinary check-ups at the children's welfare clinics.

Case 30. F.G., a girl, was suspected Pi Z on the screen. She was delivered in the 31st gestational week by a caesarian section because of bleeding placenta praevia. Apgar score 2 p. at 1 minute and 7 p. at 5 minutes. B.w. 1,200 g. She had apnoeic spells during the first day but then was well until 3 weeks of age, when she got septicemia, which was successfully treated. Check-ups at 1/4 and 1 year of age revealed nothing remarkable.

Case 31. M.G., a girl, was suspected Pi Z on the screen. She died at 2 months of age from heart malformation and staphylococcal septicemia. Autopsy showed mild fibrosis of the liver and proliferation of the bile ducts.

Summary of the clinical results of the Pi Z children:

GROUP I A. Of the 122 Pi Z infants, 9 developed prolonged obstructive jaundice with severe clinical symptoms. All were Pi Z, 6 of these 9 were boys and of them 3 had heredity for liver disease; 2 had siblings Pi Z who had had neonatal hepatitis and 1 an uncle who died of cryptogenic cirrhosis. The number of SGA infants, 4 out of 9, was increased. 1 pregnancy was complicated by insufficiency of the placenta. One child developed hyperbilirubinemia within the first few days of life; another, septicemia and hyperbilirubinemia; and a third symptomatic hypoglycemia. The infants were admitted to hospital within the first 6 weeks of life because of jaundice, poor weight gain and often pale-coloured stools. The conjugated S-Bil ranged from 4.7 to 12.0 mg/100 ml and returned to normal within 4 months in all of them. Serologic examination for virus during the acute phase in 6 of the 9 infants was negative. Check-ups of all 9 children at 1-2 years of age revealed hepatomegaly in 2, increase of S-ALP and S-GPT in 2 and neurological abnormalities in 1 of them, abnormal S-GPT only in 3, and nothing remarkable in 2. At check-ups of 3 children at 2-3 years of age 1 showed nothing remarkable, 1 had abnormal S-GPT and 1 had clinical signs of liver cirrhosis and a persistent cough.

GROUP I B. All 5 Pi Z infants of this group had biochemical signs of cholestasis but generally without any clear clinical symptoms. None had heredity for liver disease. 4 of them were boys. 3 were SGA. The abnormalities of the liver tests were detected at routine check-ups. The exact duration of jaundice is therefore not properly known. Within about one month all of the children were healthy. Serologic examination for virus was done in 2 of the infants and was negative in both. At 1-2 years one of the five children developed hepatosplenomegaly with an increase of both S-ALP and S-GPT. Two had normal and two abnormal S-GPT, while all 4 were apparently healthy. Check-up of 4 at 2-3 years showed clinical and biochemical evidence of the development of cirrhosis in 1, and no-

thing remarkable in the others.

GROUP II. Liver disease without jaundice was suspected clinically in 8 children, all Pi Z. 5 were boys. 3 pregnancies were complicated by benign cholestatic jaundice. 3 were SGA. 1 child had hypoglycemia and 2 hyperbilirubinemia with a maximal level during the 1st week. But the jaundice gradually subsided and was recognized by the mother when the child was at least 1 month and 2-3 months old. Conjugated S-Bil was 1.5 mg/100 ml and S-GT 251 U/l in one of them and S-Bil normal, while S-GT was 260 U/l and S-GPT 265 U/l in the other. Umbilical bleeding and prolongation of the prothrombin time were the initial symptoms in a 3rd child. The other 5 children included 1 with hepatomegaly, 1 with hepatosplenomegaly, 1 with splenomegaly and failure to gain weight, 1 with failure to gain weight and 1 with hepatomegaly discovered at 6 months. The S-GT was increased in all. 1-2 years old all were apparently healthy, 1 had hepatomegaly and abnormal S-GPT and another 3 had abnormal S-GPT. Check-up of 6 at 2-3 years revealed abnormal S-GPT in 2. All 6 were apparently healthy.

GROUP III. 98 infants with no clinical symptoms of liver disease were followed up. Only one child had heredity of liver disease -an uncle who had died of hepatoma. 7 gestations and 4 of the deliveries were complicated. 8 of the infants were preterm. 17 were SGA. The S-GT at 3 months and S-GPT at 3, 6 and 12 months of age are given in Fig. 5. 62 of the infants were reviewed 1-2 years and 34 at 2-3 years of age. At 2-3 years S-GPT was abnormal in 6 of the 19 hitherto examined. One child with morbilli, perforative appendicitis and fatty liver, has shown clinical symptoms of liver disease. The others have been apparently healthy except those with respiratory disease (Table 10), 1 who had a severe anaphylactic shock and 1 with congenital chondrodystrophy.

Summary of the clinical results of the Pi SZ children:

GROUP III. All the 48 Pi SZ infants belonged to this group. None had any heredity for liver disease, 5 gestations were complicated as was 1 delivery. 3 infants were preterm and only 2 SGA. 4 had hyperbilirubinemia within the 1st week, and in 1 of these infants hyperbilirubinemia clinically was of long duration but disappeared within 2 months. Another had symptomatic hypoglycemia. 1 child died a sudden unexpected infant death. The results of the liver tests at 3, 6 and 12 months of age are given in Fig. 5. 47 were followed up 1-2 years and 20 of them for 2-3 years. So far only 5 of them have been subjected to liver tests at 2-3 years of age. The results of S-GPT were normal in 4 and 50 U/l in one. 1 child had respiratory symptoms, 1 monoarthritis and 1 a severe malformation of the hand while the others were apparently healthy.

Summary of the clinical results of the Pi- and Pi S- children:

2 Pi Z- and 1 Pi S- girls were found. 1 Pi Z- child had abnormal S-GPT examined twice during the 1st year, while the results of the liver tests in the other 2 were normal. All 3 were apparently healthy at 2-3 years of age.

The liver disease in α_1 -AT deficient (Pi Z) children presented different types of functional derangements; cholestatic, cytotoxic and mixed variants. Prolonged obstructive jaundice with severe clinical symptoms occurred in 7% of the Pi Z infants, a further 3% had sub-clinical jaundice discovered at routine controls and 6% had various clinical symptoms of liver disease. A raised level of conjugated bilirubin, however, is a late manifestation of cholestasis and inflammatory changes in the bile ducts and hepatocytes may occur before or in the absence of hyperbilirubinemia. S-GT is a more sensitive biochemical parameter of cholestasis and was increased not only in those infants with clinical symptoms of liver disease, but also in about half of the asymptomatic Pi Z infants within the first few months of life. None of the Pi SZ infants had clinical symptoms of

liver disease and only about 10% had a slightly increased level of S-GT. The high frequency of abnormal S-GPT's in the Pi Z children, about half of the S-GPT's above 50 U/l during the first year, suggests damage of the hepatocytes and probably an increased cell turnover. In the Pi SZ children, however, less than one tenth of the S-GPT's were only slightly increased.

Severe cholestatic liver disease shows a striking tendency to occur in certain families (92) and 3 of the 9 in this study had positive family histories, indicating another important genetic factor. The proportion of boys to girls was about 2:1 in the children with clinical or biochemical signs of cholestasis. This is in agreement with earlier observations in neonatal hepatitis and different from the sexdistribution in biliary atresia where the number of girls exceeds that of boys (93). The pathophysiologic background of these findings is obscure, but suggests a hormonal influence upon the cholestatic reaction in these infants.

The number of SGA infants among those with symptoms of liver disease, 10 out of 22, was increased. This means that a considerable number of the infants with cholestasis might have been affected already during intrauterine life. The concentration of α_1 -AT is equal in intrauterine and in adult life, but the other major protease inhibitor, α_2 -M, increases from about 75% below during the 2nd trimester to about 50% above in the adult level at term. Sinusoidal endothelial cells provide larger fenestrations than do capillaries elsewhere, and thereby probably affects the distribution of protease inhibitors, especially α_2 -M, between the intra- and extravascular spaces. It is not known with certainty whether endo- or exogenous factors are capable of injuring the bile ducts and hepatocytes or whether α_1 -AT is of importance for protecting the liver tissue against proteolytic enzymes. A primary protective role of α_1 -AT may account for the fact that liver disease has seldom been reported in children of the Pi SZ phenotype (71, 72). Our results suggest that a α_1 -AT level above 40-50% is of importance to protect the liver tissue.

Even in the prospective English study of neonatal hepatitis syndrome no etiological or associated factor could be identified in about 50% of the cases (67). Genetic factors, mainly α_1 -AT deficiency, chromosomal abnormalities, blood group incompatibility and infections, were important etiological factors in the remaining half of these children. External factors, such as perinatal stress and neonatal infections have been suspected to precipitate liver disease, but most workers, like ourselves, have found no noteworthy association between hepatotropic viral agents and the liver disease in the Pi Z infants (54, 64, 65, 94). The perinatal histories of those Pi Z infants who were severely affected by liver disease were rather uneventful compared with those who were clinically healthy. We examined the infants serologically for virus, including hepatitis B antigen in 6 out of 9 children with prolonged obstructive jaundice and the results were negative.

The role, if any, of monohydroxy bile acids, in the generation of cholestasis in the newborn is obscure (95, 96). Animal experiments have shown that monohydroxy bile acids may have cholestatic and inflammatory effects (97) and interfere with bile formation and indirectly reduce the elimination of bilirubin. The bile acid metabolism in newborns differs from that in adults, and monohydroxy bile acids occur in various amounts in the meconium (98). These toxic bile acids are detoxified by sulphate conjugation (99). The sulphation enzymes of the liver microsomes may be less active in the neonates, and it has been suggested that the cholestasis may be the result of hypoactive smooth endoplasmic reticulum of the hepatocytes that produces an excess of monohydroxy bile acids (100). With time, sulphation as well as the production of di- and trihydroxy bile acids that form soluble complexes with the monohydroxy bile acids may increase. These events may stimulate the canalicular bile flow and indirectly reduce the intensity of jaundice. But so far no metabolic error in bile acid synthesis or detoxifying mechanisms have been established and whether the liver in α_1 -AT deficiency might be more vulnerable is unknown.

A principal function of α_1 -AT may be to inactivate proteolytic enzymes escaping from the cellular compartments of the granulocytes and macrophages (101). The protease inhibitory capacity is markedly diminished in severe α_1 -AT deficiency (Pi Z) so that, theoretically, proteolytic enzymes may reach the hepatocytes and bile ducts in the free form and act as hepatotoxins, either directly, or indirectly by the formation of hepatotoxic metabolites. The liver is the largest reticuloendothelial organ, and the liver macrophages, the so called Kupffer cells, constitute about 10% of the total cells of the liver (102). These phagocytes may be exceptionally active during the early months of life. Factors contributing to the problem may include, among other things, bacterial colonization of the bowel with formation of antigen-antibody complexes and **release of endotoxins**, low γ M- and γ A-globulin levels and decline in the level of γ G-globulin. In some infants the susceptibility of the bile ducts may be unique or hepatocanalicular toxins produced which, at least theoretically, may account for their cholestasis. Both of the alternatives may have a genetic background.

It is unlikely that the liver disease results directly from the presence of inclusion bodies in the hepatocytes, because such material probably occurs in all subjects with the Pi^Z gene, but liver disease only infrequently. On the other hand, it is surprising that none of the Pi-- individuals have shown any signs of liver fibrosis or cirrhosis (35, 103). Even electron microscopic examination of the liver in one of them revealed nothing remarkable. Most of the inclusion bodies are found in the periportal area. The flow of bile toward the portal areas is opposite to that of sinusoidal plasma toward the central vein and therefore the concentration of hepatotoxic agents should be highest around the periportal hepatocytes. A toxic effect on the endoplasmic reticulum may further decrease the already sluggish movement of α_1 -AT synthesis in Pi Z subjects and favour the formation of inclusion bodies.

In conclusion, prolonged obstructive jaundice occurs in 7% of the Pi Z infants whereas 3% have subclinical symptoms and 6% a va-

rious symptomatology. About half of the remaining Pi Z infants had biochemical abnormalities suggesting injury of the hepatocytes or bile ducts. None of the Pi SZ infants had liver disease and few of them slight abnormalities of the liver tests. This fact suggests that a level of α_1 -AT above 40-50% of the normal may be critical. Severe cholestasis occurs in certain families and two thirds of the infants with clinical or biochemical evidence of cholestasis were boys, suggesting another genetic factor and hormonal factors of pathogenetic importance.

α_1 -antitrypsin deficiency and lung disease in children 0-3 years of age (unpublished observations)

In our study a special inquiry was made for upper respiratory infections at 1/2, 1 and 2 years of age. The children with recurrent (more than twice a year) episodes of bronchitis with wheezing and dyspnea, persistent cough (more than half a year), and with recurrent croup and with pneumonia are given in Table 10 and the child with pneumonia is described in a case report below. No control group was available but the number of children with evidence of respiratory disease is not impressive. So far, about two thirds of the Pi Z and SZ children followed up are over one year and the rest over two years of age.

Table 10. RECURRENT BRONCHITIS WITH WHEEZING OR PERSISTENT COUGH, RECURRENT CROUP, AND PNEUMONIA IN Pi Z AND Pi SZ CHILDREN DURING THE FIRST TWO YEARS OF LIFE.

N = number

Symptoms	N	N
	Pi Z	Pi SZ
Recurrent bronchitis	7	1
Persistent cough ¹⁾	2	-
Recurrent croup	3	-
Pneumonia	1	-

¹⁾ Clinically suspected liver cirrhosis in both.

Case report. P.H., a boy (b.w. 3,140 g), was the 5th child, parents and siblings were healthy. Jaundice appeared within the first two days with transient occurrence of unconjugated S-Bil 14.0 mg/100 ml on the fourth day. Pi typing showed α_1 -AT deficiency Pi Z, concentration 16%. The boy was healthy, and the liver tests within normal limits. At 8 months he had an attack of gastroenteritis and upper respiratory tract infection. S-Bil and S-ALP were normal and S-GPT 63 U/l. At 1.2 years he had bilateral bronchopneumonia with dyspnea that promptly responded to penicillin. S-Bil, S-ALP were normal and S-GPT 107 U/l. At about 2 years of age, the boy had bronchitis and fever followed one week later by rising temperature, abdominal pain and cough. Chest X-ray revealed intensive lobar pneumonia and the liver and spleen were enlarged to the iliac crest. WBC 20,000, S-Bil 1.0 mg/100 ml, GPT 41, prothrombin time decreased to 17%. During the first few days he suffered from severe dyspnea. He received large doses of penicillin i.v. for 2 weeks. After 10 days repeat X-ray showed reduced but persisting pleuropneumonia and a cystic liquefaction in the right lower lobe. The prothrombin time was normal, liver and spleen almost of normal size and S-GPT 64 U/l. 4 months after the acute episode the boy was asymptomatic, liver and spleen within normal limits, lung cyst still roentgenographically demonstrated. A liver biopsy specimen (Menghini needle) showed broadening of the portal zones. Many liver cell fragments were surrounded by thin septa. No fat accumulation, no biliary stasis and a few PAS positive inclusion bodies were seen.

The range of variation of age at onset of clinical lung disease in α_1 -AT deficiency is wide. Some components of the multifactorial background that might perhaps influence the degeneration rate of the lung tissue are known. The major protease inhibitors, α_1 -AT and α_2 -M, differ in their distribution between the intra- and extravascular spaces, 40% of α_1 -AT and about 20% of α_2 -M occur extravascularly. In children, however, the α_2 -M level is 2-3 times as high as that in the normal adult and can probably to some extent compensate for a low α_1 -AT level during childhood. The role and function of the other protease inhibitors are obscure, but it is quite possible that they influence the age of onset and progression of the emphysematous lesions. The key function of α_1 -AT is the inhibition of leucocyte proteases. Complex formation has been demonstrated with elastase and collagenase. The production and turnover of leucocytes is substantial, as mentioned before, and aging leucocytes adhere to the lung capillaries, sequester and disintegrate

and release the proteolytic enzymes into the extravascular space (104, 105). The most severe emphysematous lesions in α_1 -AT deficient patients are seen in the lung bases where the perfusion is largest and consequently where the largest amount of proteases are deposited. Excessive elastolytic activity, caused by free leucocytal elastase in α_1 -AT deficient subjects, damage the elastin fibres of the lung tissue (106).

The protease inhibiting capacity of nasal and bronchial mucus is to 10-20% related with α_1 -AT and 80-90% is due to a low molecular weight (LMW) inhibitor, molecular weight about 14,000 daltons (107). The LMW inhibitor cross-reacts immunologically with I- α -I. The inhibition of leucocyte proteases by LMW inhibitor corresponds to that of α_1 -AT. The amount of LMW inhibitor in bronchial mucus may normally be sufficient to protect the alveoli and bronchi from elastase and other proteases. When the number and activity of leucocytes and macrophages increase, α_1 -AT is probably also necessary to protect the lung tissue from elastolytic activity.

Acute inflammatory processes in the lungs are generally followed by an increase in acute phase proteins, including α_1 -AT. The inflammation causes a shift in the inhibitors to the extravascular space. The proteolytic enzymes normally contribute to the cleaning up of the infectious process. Such cleaning, however, becomes harmful when the capacity of the inhibitors is insufficient and free proteolytic enzymes attack elastic fibres and alveolar membranes. Other serum protease inhibitors and LMW inhibitor probably compensate for low α_1 -AT in most circumstances, but α_1 -AT deficient persons may run an increased risk of tissue destruction in pneumonia.

Certain external factors, such as cigarette smoke and air pollution probably influence the onset and progression of emphysema in persons with α_1 -AT deficiency. The number and activity of the alveolar macrophages is considerably increased in smokers and may influence the amount of proteases (108). The amount of α_1 -AT in

the alveolar lavage fluid and macrophages is substantially increased. Chronically increased alveolar α_1 -AT levels may be essential for the protection of lung tissue against proteolysis in cigarette smokers (109). Smoking may therefore theoretically considerably contribute to the development of emphysema especially when associated with low α_1 -AT levels. Exposure of infants to cigarette smoke influences the risk of attacks of upper respiratory disease and may thus be of importance in α_1 -AT deficient children (110).

The complexity of the biological events and inter-relationship between the factors described are considerable. That the amount of proteolytic enzymes in the leucocytes was inherited and that the early onset emphysema correlated not only with the level of α_1 -AT but also with the level of leucocyte proteases has been put forward (111), but not confirmed (112), and the hypothesis remains controversial. A regulator of the inflammatory response, the chemotactic factor inactivator, may also be deficient in α_1 -AT deficient sera (113).

SPECULATIONS

No specific long-term treatment for α_1 -AT deficiency Pi Z or SZ is available. The protease inhibitory capacity may be increased either by synthetic protease inhibitors or purified α_1 -AT concentrates. But α_1 -AT has a short survival time, which makes long-term substitution impractical. Attempts to stimulate the synthesis and secretion of α_1 -AT in the Pi Z individuals by estrogen, corticosteroids or phenobarbital have been unsuccessful. The wide normal variation of the white blood cell count may be due to different production and turnover rates. If the number of ageing leucocytes that adhere to the basal parts of the lungs might be diminished, the increased elastolytic activity in the lungs of the Pi Z subjects might be favourably affected.

Screening for α_1 -AT deficiency in early life provides a rea-

sonable possibility of selecting individuals who should as far as possible avoid exposure to smoke, polluted air. Vaccination against whooping cough, measles and influenza and prompt treatment of pulmonary infections with antibiotics should be recommended. Since the anti-smoking propaganda has proved inconclusive, the identification of persons in whom cigarette smoke is apt to induce obstructive lung disease and counselling them not to start smoking, seems a major prophylactic importance. In addition, exposure of an infant to smoke from the burning end of a cigarette and exhaled smoke offers serious threat to the lungs of the child. Parents who smoke at home can increase the frequency of respiratory tract disease and should be advised to stop smoking.

Since the pathogenesis of liver disease is still puzzling, the possibilities of intervening are only theoretical. The level of α_1 -AT seems to be a major factor and the administration of α_1 -AT during the first months of life, at least to high risk families in which earlier children had severe liver disease, might be useful. The recipient may, however, develop antibodies against α_1 -AT. This is a reasonable probability and serious drawback, because all the various phenotypes probably have differences in the peptide. If further investigation reveals that monohydroxy bile acids are of importance, administration of enzyme-inducing agents or chole-retic bile salts might be tried to mitigate the cholestatic effect.

Intrauterine diagnosis from amniotic fluid will probably be impossible because α_1 -AT is mainly of maternal origin (114). Examination of blood samples from the fetus seems the only possibility of making a prenatal diagnosis in Pi Z families in which earlier children have had severe congenital liver disease.

Screening for α_1 -AT deficiency, one of the most common metabolic diseases in Sweden, is seriously considered. Severe α_1 -AT deficiency Pi Z and SZ occurs in about 1 in 500 subjects. Wilson and Jungner (115) stated the WHO principles for screening of metabolic diseases. Using these criteria it is doubtful whether screening should be done for any inherited disease except phenylketon-

uria (116). α_1 -AT deficiency does not qualify for mass-screening if all the WHO criteria should be met. The only treatment presently available to prevent emphysema is to warn α_1 -AT deficient individuals against smoking. We feel, however, that long-term treatment will be available in the near future. The benefits to be gained by prophylaxis against certain upper respiratory tract diseases, such as measles, whooping cough and influenza, and early antibiotic treatment may theoretically be substantial. The environment of the child may be further improved by advising parents who are heavy cigarette smokers, to stop smoking. Important factors possibly contributing to the development of liver and lung disease are, however, unknown. The question as to what degree Pi SZ subjects are predisposed to obstructive pulmonary disease is important and still unanswered. The outcome of severe α_1 -AT deficiency in a given case is unpredictable.

Serious consideration must be given to the question as to the age the α_1 -AT screening should be done. The optimal prophylactic effect will probably be gained if screening is done in newborns when our method provides a reliable and cheap screening test. These factors must be balanced against the possible harmful psychological effects to the family and child in the different ages, effects that may outweigh the benefits. Our hypothesis, like that of other workers (47), is that screening of persons at risk in certain occupations and children should be recommended and subjects with severe α_1 -AT deficiency identified before they start smoking or train for jobs in polluted environments. Statistically, there is no refuge for Pi Z smokers and they should know it (117).

GENERAL SUMMARY

This study comprises a prospective investigation of children with α_1 -antitrypsin (α_1 -AT) deficiency found on screening 200,000 Swedish infants. Special attention has been given to the variation of protease inhibitors in fetuses and newborn infants, to the screening test and to the prevalence of α_1 -AT deficiency genes as well as to the occurrence of liver and lung disease.

Low levels of protease inhibitors were found on the 1st day of life in infants with hyaline membrane disease. The concentration of α_1 -AT and antichymotrypsin (Achy) were significantly reduced and increased to normal on the 2nd day. In fetuses α_1 -AT was normal, Achy 2% and α_2 -macroglobulin 1/3 of the normal adult level. The protease inhibitors studied were increased in infants born after premature rupture of the fetal membranes.

The screening test for α_1 -AT deficiency, a semiquantitative electroimmunoassay using a dried drop of blood on filter paper, was reliable. Probably all infants with α_1 -AT deficiency Pi Z were found, while about 2/3 of the Pi SZ subjects were excluded with the cut-off level of 40% of the reference.

The Pi^Z gene frequency was 0.026 and the Pi^- gene frequency about 0.0002.

200,000 newborn infants were screened for α_1 -AT deficiency. 120 were Pi Z, 48 Pi SZ, 2 Pi Z-, 1 Pi S- and 19 suspected of α_1 -AT deficiency on the screen have not been Pi typed.

9/120 Pi Z infants had prolonged obstructive jaundice with severe clinical symptoms. At 1-2 years of age, 2 had hepatomegaly, 2 increase of S-ALP and S-GPT and in one of them neurological abnormalities, 3 abnormal S-GPT only and 2 showed nothing remarkable. At 2-3 years 3 children were examined; 1 had clinical signs of liver cirrhosis, 1 abnormal S-GPT and 1 showed nothing remarkable.

5/120 Pi Z infants had prolonged jaundice with subclinical symptoms. At 1-2 years 1 had hepatosplenomegaly and increase of both S-ALP and S-GPT, 2 had abnormal and 2 normal S-GPT, while all 4 were apparently healthy. At 2-3 years 4 have been followed up; 1 had bio-

chemical and clinical suspicion of cirrhosis, and 3 showed nothing remarkable.

8/120 Pi Z infants had suspected liver disease without jaundice at the clinical check-up. 1-2 years all were apparently healthy, but 1 had hepatomegaly and abnormal S-GPT, and 3 abnormal S-GPT only. Check-up of 6 at 2-3 years revealed abnormal S-GPT in 2, and all 6 were apparently healthy.

98/120 Pi Z infants had no clinical liver disease, although liver tests were abnormal in about half of those examined at 3, 6 and 12 months of age. At 2-3 years S-GPT was abnormal in about 1/3 of the 19 hitherto analyzed. 1 child had symptoms of liver disease, 2 years old, in connection with morbilli and perforative appendicitis. The others have shown no clinical signs of liver disease.

None of the 48 Pi SZ children had clinical symptoms of liver disease. 10/42 had abnormal liver tests at 3 months, while only 1 of those examined at 6 and 12 months had abnormal S-GPT. 1 child died a sudden unexpected infant death. None have any clinical signs of liver disease when half have been followed up 1-2 years and half 2-3 years.

2 Pi Z- and 1 Pi S- children were found. 1 Pi Z- girl had abnormal S-GPT twice during the 1st year, while liver tests of the other 2 were normal. All 3 were healthy at 2-3 years of age.

3 of the 9 Pi Z infants with severe cholestasis had positive family histories for liver disease. The number of small for gestational age infants was significantly increased among the Pi Z infants with clinical signs of liver disease. 2/3 of the Pi Z infants with clinical or biochemical signs of cholestasis were boys.

A special inquiry for respiratory disease was made. 7 Pi Z and 1 Pi SZ children had bronchitis with wheezing more than twice a year. 2 Pi Z children with coexisting cirrhosis had persistent cough for more than half a year. 3 Pi Z children had recurrent croup. 1 Pi Z child had severe pneumonia with a cystic liquefaction. Since no control group was available the results are inconclusive, but the number of α_1 -AT deficient children with severe

respiratory disease during the first three years of life is not impressive.

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